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Biocompatible chiral monolithic stationary phase synthesized via atom transfer radical polymerization for high performance liquid chromatographic analysis

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ABSTRACT

Novel biocompatible chiral monolithic stationary phase was prepared by reverse and direct atom transfer radical polymerization (ATRP) methods. By taking advantages of the controlled/living property of ATRP method, the chiral monolith was prepared by reverse ATRP (RATRP) firstly. An attractive feature of RATRP is the prepared polymer containing a terminal radically transferable atom that can initiate another post-polymerization reaction by direct ATRP. Then, the biocompatible poly(hydroxyethyl methacrylate) (PHEMA) was grafted on the surface of the chiral monolith by direct ATRP as a diffusion barrier for proteins. This biocompatible chiral monolith was successfully used as restricted access stationary phase for determination of enantiomers in biological samples with direct injection by high-performance liquid chromatography (HPLC).

1. Introduction

Monolithic stationary phases have been widely used for HPLC analysis because of their inherent advantages such as high column efficiency and low consumption of samples [1–4]. In the past decade, various monolithic chromatographic materials have been developed including the organic polymer- and silica-based monoliths [5–7]. The monoliths based on organic polymers are typically prepared from styrene, acrylamide or methacrylate monomers, or are polymerized from ring-opening reaction [4]. Among them, polymethacrylate polymers have been well used for preparing the monolithic separation media, because their porous properties can be easily tailored [8,9]. Furthermore, some polymethacrylate-based chiral monolithic columns have been reported and received good enantio-separation performance [9–12]. However, the analysis of chiral drugs in biological fluids is still a challenging task for the organic polymer-based monolithic columns. Because, the sample pretreatment procedures were needed to remove proteins by precipitation, liquid–liquid extraction or solid phase extraction [13].

Recent years, the biocompatible chiral restricted access materials (RAMs) have been successfully utilized as chromatographic stationary phase for direct injection of biological samples [14,15]. These chiral RAMs were mainly prepared based on porous silica gel support, which were surface-modified with hydrophilic polymer networks (such as polyvinyl alcohol and albumin from bovine serum) and inner-bonded with chiral selectors (such as β-CD and glycopeptide antibiotics) [16–19]. As stationary phases for HPLC, the chiral RAMs allow the analytes penetrating into the pores of silica gel and interact with the chiral selectors bonded on the inner surface, while proteins are eluted in the void volume. In that way, the chiral drugs in biological samples can be analyzed with direct HPLC injection, and the analytical efficiency and accuracy of HPLC separations can be well improved.

Herein, for the first time, biocompatible chiral monoliths were prepared and explored as novel stationary phases for analysis of chiral drugs in biological samples with direct HPLC injection. Reverse atom transfer radical polymerization (RATRP) with excellent controllability of the molecular weight and polydispersity was employed to prepare β-CD containing polymeric monolithic columns (CD-MC) [20–23]. An attractive feature of RATRP is the prepared polymer containing a terminal radically transferable atom that can initiate another post-polymerization reaction by direct ATRP (Fig. S1). So, in present study, the direct ATRP,

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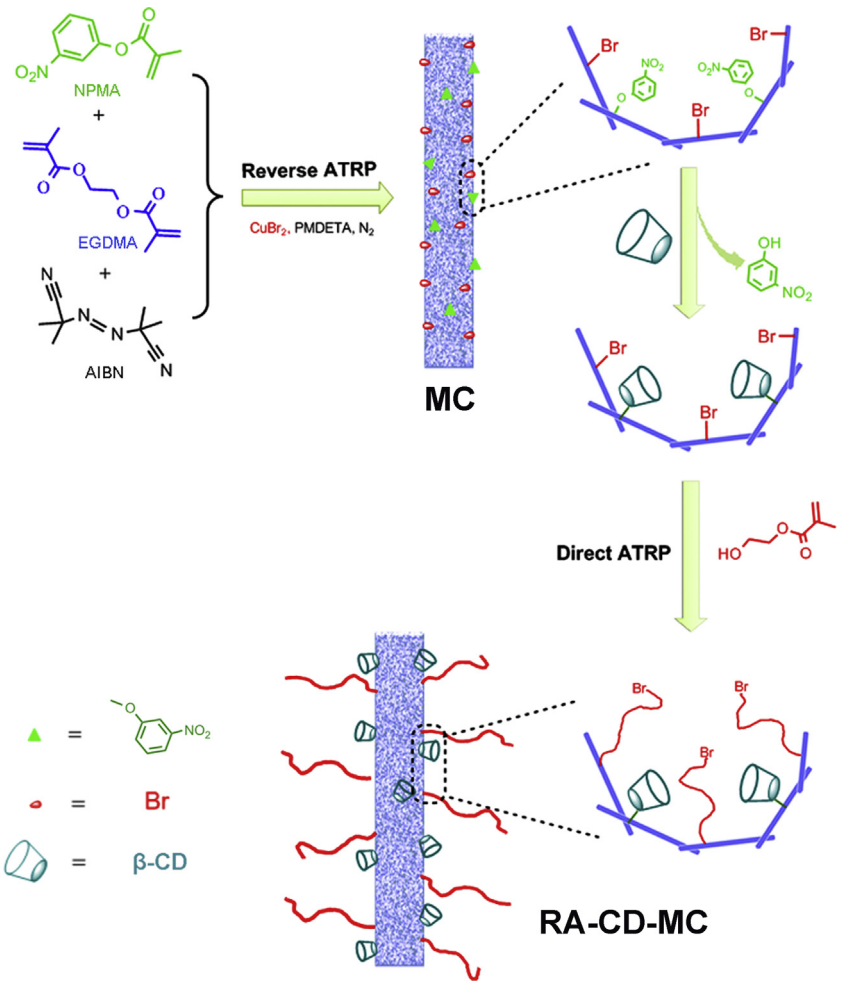

Fig. 1. Illustration of the synthesis process of RA-CD-MC.

surface-initiated from the polymeric monoliths, was employed for grafting biocompatible polymer brushes, which can significantly improve their surface hydrophilicity and acted as a protective layer to prevent proteins in biological samples from being adsorbed irreversibly on the monoliths. The chiral separation ability of the biocompatible restricted access monolithic column (RA-CD-MC, Fig. 1) was evaluated by reversed-phase HPLC method with direct injection of biological samples. And the monolithic stationary phases gave well resolution for the separation of drugs in human plasma. Meanwhile, good protein recovery was obtained.

2. Materials and methods

2.1. Reagents and chemicals

β-CD was purchased from Aoboxing Biotech. Co. Ltd. (Beijing, China). N,N,N′,N′,N″-pentamethyl diethylenetriamine (PMDETA), hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), *o*-, *m*-, *p*-nitrophenol, azobisisobutyronitrile (AIBN) and methacryloyl chloride were obtained from Sigma–Aldrich (St. Louis, USA). Bovine serum albumin (BSA) was from Solarbio Science & Technology Co. Ltd. (Beijing, China). Chlorthalidone, propranolol, aminoglutethimide, benzoin, amlodipine, chlorpheniramine, phenylalanine and ibuprofen were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 2-Bromoisobutyryl bromide (BIBB) was from Hengye Zhongyuan Chemical Co. Ltd. (Beijing, China). Triethylammonium acetate buffer solutions (TEAA solution) were prepared according to the reference [19]. All reagents were analytical grade and used as received unless otherwise stated.

2.2. Instrumentation

The HPLC system consisted of a Shimadzu LC-20 AD pump and a SPD-20A UV detector. NMR spectra were recorded on a Bruker AVANCE III instrument. The SEM images were recorded on a Hitachi S-3400N II scanning electron microscope. The IR spectra were obtained from an AVATAR-360 FTIR instrument (Nicolet, USA).

2.3. Synthesis of m-nitrophenyl methacrylate (NPMA)

NPMA was prepared by adding 5 g of acrylyl chloride to a solution of 2.5 g of potassium hydroxide and 4 g of *m*-nitrophenol in 200 mL of water at 0 °C. The product was recrystallized in ether (yield 75%). NMR: δ_H (300 MHz; $CDCl_3$; Me_4Si) 8.13–8.09 (1H, m, HAr), 8.03–8.01 (1H, t, HAr), 7.60–7.54 (1H, t, HAr), 7.50–7.45 (1H, m, HAr), 6.39 and 5.83 (2H, m, C=CH_2), 2.08 (3H, m, CH_3). δ_C (100 MHz; $CDCl_3$; Me_4Si) 164.8 (C=O), 151.1, 148.7, 130.0, 128.2, 120.8 and 117.6 (HAr), 135.1 and 128.5 (C=C), 19.4 (CH_3) (Fig. S3 and Fig. S4).

2.4. Preparation of monolithic column (MC) via RATRP

The monolithic column (MC) was prepared via in situ RATRP using a binary solvent (acetonitrile/lauryl alcohol = 9/1, v/v)